Exhibit 2

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Biochemistry

Molecular cloning and expression of cDNA encoding the enzyme that controls conversion of high-mannose to hybrid and complex *N*-glycans: UDP-*N*-acetylglucosamine:α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I

(glycoprotein/glycosylation/glycosyltransferase/polymerase chain reaction)

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ABSTRACT UDP-GlcNAc:α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I (GnT I; EC 2.4.1.101) catalyzes an essential first step in the conversion of high-mannose *N*-glycans to hybrid and complex *N*-glycans. Cloning of the gene encoding this enzyme was carried out by mixed oligonucleotide-primed polymerase chain reaction amplification of rabbit liver single-stranded cDNA using sense and antisense 20- to 24-base-pair (bp) primers. A rabbit liver library in phage λgt10 yielded a 2.5-kilobase (kb) cDNA with a 447-amino acid coding sequence. None of the nine asparagine residues were in an Asn-Xaa-(Ser or Thr) sequence, indicating that the protein is not N-glycosylated. There is no sequence homology to other previously cloned glycosyltransferases, but GnT I appears to have a domain structure typical of these enzymes—i.e., a short amino-terminal domain, a transmembrane domain, a "neck" region, and a large carboxyl-terminal catalytic domain. RNA was transcribed off the 2.5-kb cDNA, and *in vitro* translation with rabbit reticulocyte lysate yielded a 52-kDa protein with GnT I activity.

The biosynthesis of highly branched *N*- and *O*-glycans is potentially important to many biological phenomena (1–3). All *N*-glycans share the common core structure Manα6(Manα3)Manβ4GlcNAcβ4GlcNAcβ-Asn. Complex *N*-glycans have branches that are initiated by the action of Golgi-localized GlcNAc-transferases designated GnT I to VI (4). The conversion of high-mannose *N*-glycans to complex and hybrid *N*-glycans is controlled by UDP-GlcNAc:α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I (GnT I; EC 2.4.1.101), which catalyzes the reaction:

UDP-GlcNAc + (Manα6[Manα3]Manα6)(Manα3)Manβ4R →

(Manα6[Manα3]Manα6)(*GlcNAcβ2*Manα3)Manβ4R + UDP,

where R is GlcNAcβ4(+/−Fucα6)GlcNAc-Asn-Xaa.

GnT I is essential for subsequent action of several enzymes in the processing pathway (5–8)—i.e., GnT II, III, and IV require the prior action of GnT I, and GnT V and VI require the prior action of GnT II. GnT I has been reported in hen oviduct, Chinese hamster ovary cells, baby hamster kidney cells, bovine colostrum, pig trachea, and mammalian liver (5, 6, 9, 10). The enzyme has been partially purified from bovine colostrum (11), from pig liver and trachea (12), and, to homogeneity, from rabbit liver (13, 14).

We have used mixed oligonucleotide-primed polymerase chain reaction (PCR) amplification (15, 16) to clone a 2.5-kilobase (kb) cDNA coding for rabbit liver GnT I. The protein contains 447 amino acids and has a domain structure typical of glycosyltransferases—i.e., a short amino-terminal domain, a transmembrane domain, a "neck" region, and a large carboxyl terminal catalytic domain. Thus, the gene encoding a medial Golgi-localized glycosyltransferase has been cloned and the sequence determined.‖

MATERIALS AND METHODS

Preparation of Peptides. Glycerol, Triton X-100, and salts were removed from 15 μg of purified enzyme (14) by "inverse-gradient" reversed-phase HPLC (RP-HPLC) (17). The enzyme solution (100 μl) was diluted to 1.2 ml with 1-propanol and loaded on a VeloSep C_8 cartridge (3-μm particle size, 30 × 2.1 mm i.d.; Applied Biosystems) equilibrated in 100% 1-propanol at 40°C. GnT I was eluted at 0.1 ml/min by a linear gradient (5%/min) of decreasing 1-propanol concentration (100–50%) generated with 100% 1-propanol and 50% 1-propanol/50% water containing 0.4% (vol/vol) trifluoroacetic acid at 40°C. The GnT I-containing fraction was adjusted to 0.02% (wt/vol) with respect to Tween 20 (Pierce), concentrated to 100 μl under vacuum, and diluted to 1.5 ml with 5% (vol/vol) formic acid containing 0.02% Tween 20.

Edman degradation of purified GnT I (~200 pmol) yielded no amino-terminal sequence. GnT I was digested with pepsin (Sigma) at an enzyme/substrate mass ratio of 1:20 for 1 hr at 37°C, and the digest was fractionated by RP-HPLC to yield peptides 5 and 6 (Fig. 1). Core GnT I remaining after pepsin digestion was reduced with dithiothreitol and alkylated with iodoacetic acid (18) to give core S-carboxymethylated(SCM)-GnT I, which was purified by RP-HPLC (18, 19). Pepsin-treated core SCM-GnT I (10 μg in 1 ml of 1% ammonium bicarbonate/1 mM $CaCl_2$/0.02% Tween 20) was digested with trypsin (Worthington) at an enzyme/substrate mass ratio of 1:20 for 16 hr at 37°C. Trypsin resulted in little further digestion of the pepsin-treated material. Sequence analysis of a portion of this material resulted in 33 amino acid assignments (peptide 1 in Fig. 1). Pepsin and trypsin-treated core SCM-GnT I (8 μg in 1 ml of 1% ammonium bicarbonate/0.02% Tween 20) was digested with thermolysin (Sigma) at an enzyme/substrate mass ratio of 1:20 for 2 hr at 50°C, and the digest was fractionated by RP-HPLC to yield peptides 2, 3, 4, 7, and 8 (Fig. 1).

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Abbreviations: GnT I, UDP-GlcNAc:α3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I; PCR, polymerase chain reaction; RP-HPLC, reversed-phase HPLC; R, GlcNAcβ4(±Fucα6)GlcNAc-Asn-Xaa.

‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M57301).

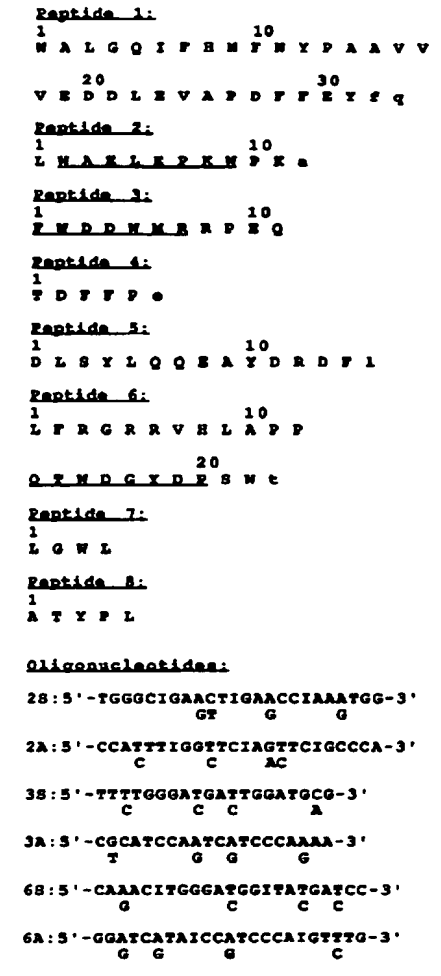

FIG. 1. Amino acid sequence data for the eight peptides isolated from rabbit liver GnT I and nucleotide sequences of the six synthetic oligonucleotides prepared on the basis of the peptide sequences. Single letter code is used for amino acid sequence data; upper case letters indicate firm assignments, and lower case letters indicate tentative assignments. Underlined sections of the peptide sequences indicate regions used for the design of oligonucleotide probes. Probes 2, 3, and 6 were based on peptides 2, 3, and 6, respectively; S indicates "sense" and A indicates "antisense" directions.

HPLC. RP-HPLC was carried out on a Hewlett–Packard liquid chromatograph (model 1090A) fitted with a diode array detector (model 1040A) (18). A Brownlee RP-300 column (30-nm-pore-size, 7-μm-diameter dimethyloctylsilica particles packed into a stainless steel cartridge, 30 × 2.1 mm i.d.; Brownlee Laboratories) was used for all peptide separations.

Amino Acid Sequence Analysis. Automated amino acid sequence analysis of GnT I and derived peptides was performed with Applied Biosystems sequencers (models 470A and 477A) equipped with on-line phenylthiohydantoin-conjugated amino acid analyzers (model 120A). Polybrene (20) was used as a carrier.

Oligonucleotides and cDNA Synthesis. Oligonucleotides were synthesized on a Pharmacia automated oligonucleotide synthesizer at the Hospital for Sick Children–Pharmacia Biotechnology Service Centre. Total RNA was prepared from rabbit liver by the method of Chirgwin *et al.* (21, 22). Poly(A)$^+$ RNA was prepared by oligo(dT) chromatography (23). Single-stranded cDNA synthesis was performed by using the RiboClone cDNA synthesis system (Promega), total rabbit liver RNA (20 μg) as template, and a mixture of the three antisense oligonucleotide primers 2A, 3A, and 6A (Fig. 1) at concentrations of 50 nM each as primers.

Amplification of cDNA. PCR was carried out in a total volume of 0.1 ml containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, each of the four dNTPs at 0.2 mM, 0.5 μM of each oligonucleotide in six paired combinations of oligonucleotide primers (2S, 3A; 2S, 6A; 3S, 2A; 3S, 6A; 6S, 2A; and 6S, 3A; Fig. 1), 10 μl of RNA-free rabbit liver cDNA (see above), 2.5 units of *Thermus aquaticus* (*Taq*) polymerase (Perkin–Elmer/Cetus), and 0.1 ml of mineral oil. The samples were subjected to a temperature-step cycle of 94°C (0.5 min), 50°C (1 min), and 72°C (2 min) for a total of 40 cycles, followed by a 10-min extension at 72°C after the final cycle. DNA from the PCR reactions showed two products (0.45 and 0.50 kb), which were blunt-end-ligated into the *Sma* I site of pGEM-7Z (Promega) (16). The recombinant plasmids were amplified in *Escherichia coli* XL1-blue cells and purified.

Screening of Rabbit Liver cDNA Library in λgt10. The pGEM-7Z/0.5-kb PCR product plasmid was cut with *Bam*HI and used to generate a riboprobe with the Promega Riboprobe Gemini II core system. Twenty-five microliters of reaction mixture contained 32 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM spermidine, 8 mM sodium chloride, 8 mM dithiothreitol, 40 units of RNasin, 0.4 mM each of ATP, GTP, and UTP, 5 μl of [α-^{32}P]CTP (800 Ci/mmol; 1 Ci = 37 GBq), 1 μg of *Bam*HI-cut pGEM-7Z/PCR product plasmid, and 2 units of T7 RNA polymerase. Incubation was at 40°C for 2 hr. RNase-free DNase I (10 units) was added, followed by incubation at room temperature for 15 min. Buffer (80 μl of 50 mM Tris·HCl, pH 7.4/4 mM EDTA/300 mM NaCl/0.1% SDS) and tRNA (20 μg) were added, followed by extraction with phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol). The labeled RNA probe was desalted over a Sephadex G-50 column (NICK column, Pharmacia) and used to screen 10^6 plaques of a rabbit liver cDNA library in λgt10 (5′-stretch, no. TL1006a from Clontech, *Eco*RI cloning site) (24). The largest cDNA insert obtained on *Eco*RI digestion was 1.6 kb; it was subcloned into pGEM-7Z (Promega) (24), and plasmid DNA was purified (22). The cDNA library was rescreened with a 80-bp riboprobe from the 5′ end of the 1.6-kb clone. The largest cDNA insert (3.0 kb) was subcloned into pGEM-7Z.

DNA Sequencing. Two colonies of the pGEM-7Z/PCR-product recombinant plasmid (see above) containing inserts in opposite directions were sequenced directly by the single-strand dideoxynucleotide-chain-termination method (25) using deoxyadenosine 5′-[α-(^{35}S)thio]triphosphate, Sequenase (United States Biochemical) and the forward primer for pGEM-7Z. The 1.6- and 3.0-kb clones were sequenced by the Erase-a-Base System (Promega) and the single-strand dideoxynucleotide-chain-termination method. Both DNA strands were sequenced by using colonies in which the inserts were present in opposite directions.

RNA Hybridization. Rabbit liver poly(A)$^+$ RNA (5 μg) was denatured in 50% (vol/vol) formamide/6% (vol/vol) formaldehyde buffer at 65°C and was resolved by gel electrophoresis in a 1% agarose gel containing 6% formaldehyde. The RNA was transferred to a nitrocellulose filter, and the filters were hybridized with the ^{32}P-labeled 0.5-kb PCR riboprobe (see above) followed by autoradiography. The specific activity of the probe was about 10^6 dpm/ng, and the hybridization solution contained about 10^6 dpm/ml.

***In Vitro* Transcription and Translation.** The recombinant plasmid containing pGEM-7Z (Promega) and the 2.5-kb GnT I cDNA insert (rc2500 in Fig. 2) was cut with *Sph* I to generate linear plasmid. RNA was transcribed by using the phage SP6 RNA polymerase promoter and initiation site present in pGEM-7Z. RNA synthesis was carried out at 40°C for 1 hr in a total volume of 50 μl containing 40 mM Tris·HCl (pH 7.5);

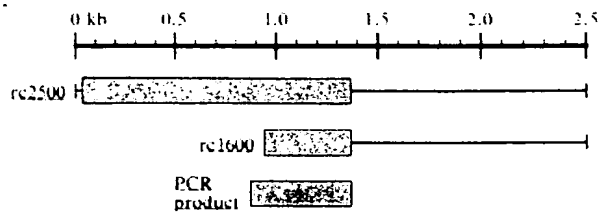

FIG. 2. Schematic representation of GnT I clones. "PCR product" is the product obtained by PCR amplification of rabbit liver cDNA; "rc1600" is the 1.6 kb-GnT I cDNA clone; and "rc2500" is the 3.0-kb GnT I cDNA clone. The shaded boxes represent the coding region. During subcloning, the 3.0-kb cDNA was reduced to 2.5 kb by a 0.5-kb deletion at the 5' end.

6 mM $MgCl_2$; 2 mM spermidine; 10 mM NaCl; 10 mM dithiothreitol; 40 units of RNasin (Promega); 0.5 mM each of ATP, UTP, and CTP; 0.1 mM GTP; 0.5 mM $m^7G(5')ppp(5')G$ (Pharmacia); 10 units of SP6 RNA polymerase; and 10 μg of linearized plasmid. Control incubations were carried out in the absence of plasmid or with a linearized pGEM-7Z recombinant plasmid containing a noncoding insert. The reaction mixture was extracted twice with phenol/chloroform/isoamyl alcohol, followed by precipitation with cold ethanol.

Protein synthesis (translation) was carried out at 30°C for 1 hr in a total volume of 50 μl containing all 20 amino acids (1 mM each), 20 units of RNasin, RNA as prepared above, and buffer and rabbit reticulocyte lysate as supplied by Promega (26). Nonradioactive amino acids were used when the products of translation were assayed for GnT I activity (see below). Separate incubations were carried out with L-[^{35}S]methionine (1000 Ci/mmol; 90 μCi per incubation) replacing nonradioactive methionine; these incubations were analyzed by SDS/polyacrylamide gel electrophoresis followed by autoradiography.

GnT I was assayed with 0.6 mM Manα6(Manα3)Manβ-hexyl (a gift from Hans Paulsen, University of Hamburg), and the product was isolated either with Sep-Pak C_{18} reverse-phase cartridges (Waters) (27) or by HPLC (4, 10).

RESULTS

Amplification of cDNA. Three amino acid sequences (Fig. 1) were chosen for the design of sense and antisense oligonucleotide primers. Deoxyinosine was substituted in positions where codon degeneracy was >2 (16). PCR was carried out with all six possible combinations of sense and antisense primers. Primer-dependent products were obtained with two of the six incubations—i.e., 2S, 6A (500 bp) and 3S, 6A (450 bp) (Fig. 3). The complete nucleotide sequence for GnT I is shown in Fig. 4.

Sequence Analysis. The 1.6-kb clone contains 0.5 kb from the 3' end of the coding region and the full 1.1-kb 3' untranslated region (rc 1600 in Fig. 2). The 3.0-kb clone yielded a 2485-bp sequence (rc2500 in Fig. 2; Fig. 4). We have shown (M.S. and H.S., unpublished data) that subcloning of the 3.0-kb DNA fragment in pGEM-7Z results in deletion of a 0.5-kb DNA fragment near the 5' end of the clone. Comparison of the cDNA sequence shown in Fig. 4 with the sequence of human genomic DNA for GnT I (unpublished data) has shown that this deleted 0.5-kb DNA fragment is not part of the GnT I gene; we do not know the origin of this DNA.

The GnT I coding sequence has 1341 bp and codes for a membrane-bound protein of 447 amino acids (M_r 52,000). There is a single hydrophobic domain (bases 62–136) flanked by charged amino acids (Fig. 4). Chou–Fasman rules (28) predict that this hydrophobic segment is capable of propagating an α-helix, as expected for a transmembrane domain.

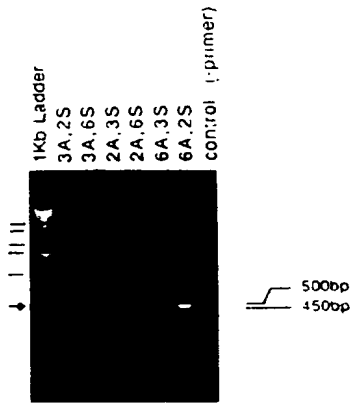

FIG. 3. Agarose gel electrophoresis (1% agarose) of the products of the PCR with rabbit liver cDNA as template and the following combinations of oligonucleotides as primers: 2S, 3A; 2S, 6A; 3S, 2A; 3S, 6A; 6S, 2A; 6S, 3A (Fig. 1). Conditions of PCR were as described. The gel was stained with ethidium bromide (0.5 μg/ml). Primer-dependent products were obtained with combinations 2S, 6A (0.50 kb) and 3S, 6A (0.45 kb). The arrow designates the 0.5 kb DNA marker; the remaining standards are at 1.0-kb, 1.6-kb, 2.0-kb, and 1.0-kb intervals thereafter.

The presumptive initiation methionine codon is at the ATG codon at position 50, which has an adenosine at position 47, thereby fulfilling the requirements for an initiation codon (29). All eight peptides shown in Fig. 1 (a total of 103 amino acid residues) can be identified in the sequence (Fig. 4); an additional five tentative assignments also match the sequence. GnT I purified from rabbit liver has a molecular mass of about 45 kDa (14). The protein has no *N*-glycans since none of the nine asparagine residues are in a typical Asn-Xaa-(Ser or Thr) sequence; we have previously shown that rabbit liver GnT I binds poorly to lectin/agarose columns (14). If there are no or few *O*-glycans, a catalytically active protein of 45 kDa can be derived by cleavage at about base position 215 (Fig. 4).

Comparison of the GnT I sequence with those of several previously cloned glycosyltransferases (30–45) revealed no sequence homology, but GnT I appears to have a domain structure typical of these enzymes (46). Searches of the GenBank nucleotide data base (release 62.0) with the coding region of GnT I and of the Protein Identification Resource, National Biomedical Research Foundation (release 23.0) with the GnT I amino acid sequence revealed no significant similarities to other sequences.

The complete sequence has a long 3' untranslated region (bases 1391–2479) containing the consensus polyadenylation signal AATAAA at position 2435 (47). Long 3' untranslated regions are typical of the known glycosyltransferase genes and may be a feature present in other Golgi-localized enzymes (16).

Northern Blot Analysis. The PCR riboprobe was used to determine the size of mRNA in rabbit liver. A major band was detected at about 3.0 kb with some smearing at lower molecular weights (data not shown), indicating that the 2.5-kb cDNA clone (Fig. 4) may not be full-length.

***In Vitro* Transcription and Translation.** Transcription of the linearized pGEM-7Z/2.5-kb GnT I cDNA recombinant plasmid followed by translation in the presence of L-[^{35}S]methionine resulted in the appearance of a strong radioactive 52-kDa band on SDS/polyacrylamide gel electrophoresis; this band was not seen in control incubations lacking plasmid or containing control plasmid (Fig. 5). The molecular weight matches the prediction for the open reading frame shown in Fig. 4. Table 1 shows the results of GnT I assays carried out

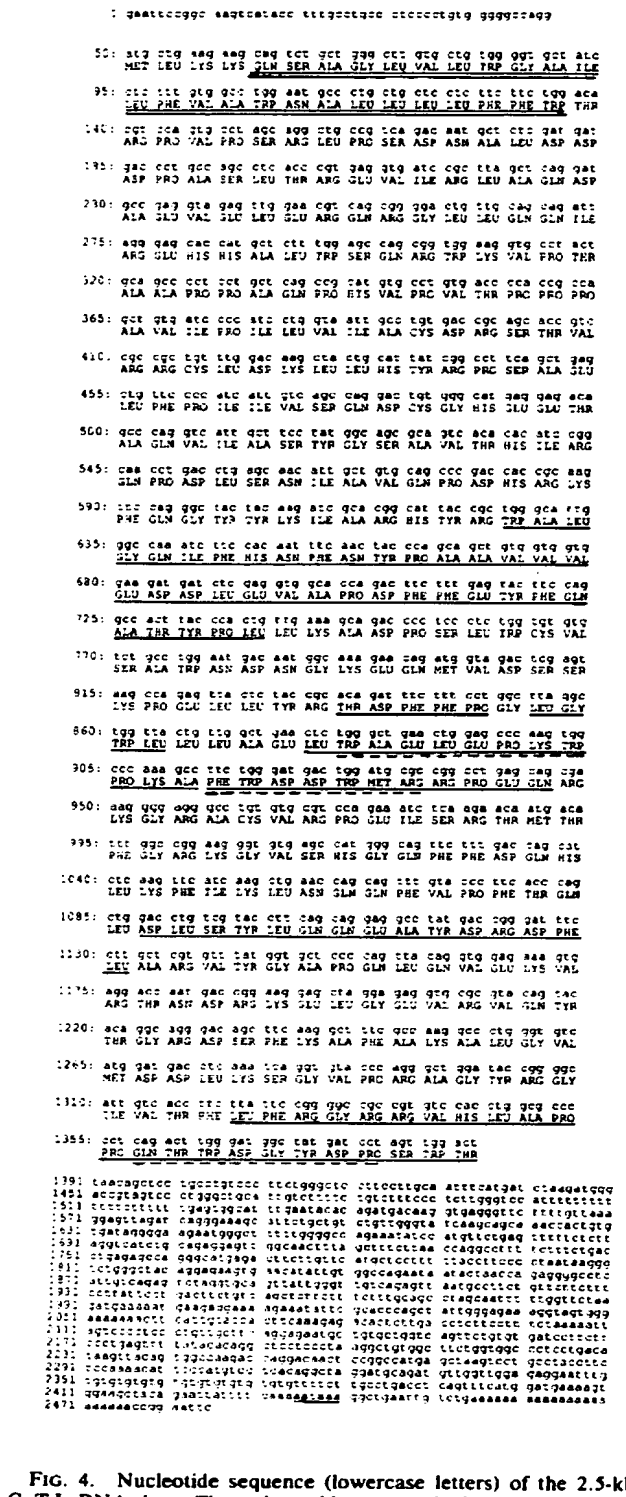

FIG. 4. Nucleotide sequence (lowercase letters) of the 2.5-kb GnT I cDNA clone. The amino acid sequence in the coding region is shown in uppercase letters. The positions of the eight peptide sequences obtained from proteolytic digests of GnT I (Fig. 1) are underlined with a single solid line; the regions of these peptide sequences used for oligonucleotide probe synthesis (Fig. 1) are additionally underlined with a discontinuous line. The putative transmembrane segment (bases 62–136) is underlined with a double line. The consensus polyadenylylation signal AATAAA at position 2435 is underlined. Only the nucleotide sequence is numbered.

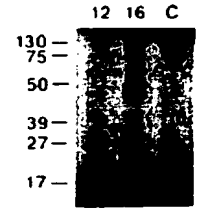

FIG. 5. An autoradiogram of an SDS/polyacrylamide gel electrophoresis experiment showing *in vitro* transcription and translation. mRNA was generated from the 2.5-kb GnT I cDNA and was used as the template for *in vitro* translation using rabbit reticulocyte lysate and L-[^{35}S]methionine. Lanes: C, no plasmid in the incubation; 12, pGEM-7Z containing the 2.5-kb GnT I cDNA with an insert between bases 56 and 57 that interrupts the reading frame; 16, pGEM-7Z containing the 2.5-kb GnT I cDNA.

on the transcription–translation incubations. The incubation containing the pGEM-7Z/2.5-kb GnT I cDNA recombinant plasmid has appreciable GnT I activity, whereas both controls show low activity. It is concluded that the 2.5-kb sequence shown in Fig. 4 can code for the synthesis of catalytically active GnT I.

DISCUSSION

GnT I catalyzes an essential first step in the conversion of high-mannose *N*-glycans to branched hybrid and complex *N*-glycans (7, 10). *In vitro* transcription/translation of the 2.5-kb cDNA reported in this paper results in GnT I activity, indicating that we have cloned the gene for the catalytic domain of this important control enzyme.

At least seven glycosyltransferases involved in the synthesis of *N*- and *O*-glycans have been cloned to date (30–45, 48). These transferases all place sugars in terminal or subterminal positions; three of them (β-1,4-galactosyl-, α-2,6-sialyl-, and α-1,3-GalNAc-transferases) have been localized to the trans-Golgi cisternae and trans-Golgi network, at least in some tissues. Most of these transferases share no significant sequence similarities but have very similar domain structures—i.e., a short amino-terminal cytoplasmic tail, a 16- to 20-amino acid transmembrane segment (noncleavable signal–anchor domain), a "stem" or "neck" region of undetermined length, and a long carboxyl-terminal catalytic domain, which is in the Golgi lumen (46).

The presence of a "neck" region is based on the finding that the α-2,6-sialyltransferase (45, 49) and the β-1,4-galactosyltransferase (31) can be cut by proteases to release a smaller catalytically active protein lacking the transmembrane domain. The exact length of this "neck" region cannot be stated with accuracy because it is not known how much of the amino-terminal sequence can be removed without loss of catalytic activity. We have shown that rabbit liver GnT I (14) and rat liver GnT II (50, 51) exist in two forms: (*i*) a large amount of presumably membrane-bound material that does not adhere to columns and has proven impossible to purify in our hands and (*ii*) a small amount of material that can be purified. In the case of GnT I, it is now clear from the sequence analysis that the 45-kDa form of the catalytically active protein that we have purified has been derived from the membrane-bound precursor by proteolytic cleavage at about base position 215 in the "neck" region (Fig. 4). Therefore, the amino-terminal blockage of this 45-kDa protein must be due to chemical modification during GnT I purification.

Rabbit GnT I; human, mouse, and bovine UDPgalactose: GlcNAc-R β-1,4-galactosyl transferases (EC 2.4.1.38); and human UDP-GalNAc:Fucα2Gal-R (GalNAc to Gal) α-1,3-GalNAc-transferase (EC 2.4.1.40) have an abnormally high number of proline residues between the transmembrane

Table 1. *In vitro* transcription–translation of rabbit GnT I cDNA

Conditions of transcription	GnT I product, nmol per total transcription incubation		
	Sep-Pak assays		HPLC 16-hr assays
	2 hr	16 hr	
No plasmid	0.04	0.21	—
Control plasmid	0.04	0.21	0.29
2.5-kb GnT I cDNA	0.41	1.05	1.32

RNA was transcribed from rabbit GnT I cDNA and translated *in vitro* as described in the text. GnT I assays were carried out on these incubations, and GnT I product was purified either by adsorption and elution from Sep-Pak C_{18} cartridges or by HPLC. Each GnT I assay contained in a total volume of 0.040 ml: 20 nmol of UDP-*N*-[1-^{14}C]-acetyl-D-glucosamine (96,000 dpm) and 24 nmol of exogenous acceptor Manα6(Manα3)Manβ-hexyl. Incorporation was corrected for control GnT I assays lacking exogenous acceptor (0.006 nmol). The activity of GnT I present in the controls was due to the presence of GnT I in the rabbit reticulocyte lysate.

domain and the catalytic domain. This proline-rich "neck" may play a role in positioning the catalytic domain in the lumen of the Golgi to enable glycosylation of glycoproteins moving along the Golgi lumen.

The domain structure of GnT I appears to be similar to that of the previously cloned glycosyltransferases. However, GnT I differs from these transferases in being a medial-Golgi enzyme, at least in some tissues (52, 53). Although no medial-Golgi glycosyltransferase has been cloned to date to our knowledge, rat liver α-mannosidase II (also a medial-Golgi enzyme) has been partially cloned (16). Comparison with GnT I reveals a 16-amino acid sequence in GnT I (Leu-His-Tyr-Arg-Pro-Ser-Ala-Glu-Leu-Phe-Pro-Ile-Ile-Val-Ser-Gln, bases 431–478, Fig. 4) that shows a high similarity score to amino acid residues 403–418 in α-mannosidase II (Leu-Gln-Tyr-Arg-Asn-Tyr-Glu-Gln-Leu-Phe-Ser-Tyr-Met-Asn-Ser-Gln).

Note Added in Proof. Preliminary reports on the cloning and structure of the genes for human and rabbit GnT I have been published (54–56). We have sequenced a 4-kb section of human genomic DNA containing a functional promoter and an intronless coding region for a 445-amino acid protein with GnT I activity. The similarity between the rabbit and human enzymes is 85% for the nucleotide coding sequences and over 90% for the amino acid sequences.

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